Claims

1. A system for in vitro producing a mammalian pre-embryo, said system comprising
   - means for obtaining a mammalian oocyte, and
   - means for obtaining a mammalian spermatozoa, and
   - an apparatus having at least two separate air-tight chambers, for which the oxygen tension of one chamber may be changed independent of the oxygen tension of the other chamber, said at least two separate air-tight chambers constitute a main chamber and at least one residence chamber,
     - said apparatus comprising at least one entrance port capable of communicating with the means for obtaining the mammalian oocyte and/or the mammalian spermatozoa, and
     - an exit port for withdrawal of the pre-embryo, as well as
     - a communication port between said at least two chambers allowing transfer of oocyte, spermatozoa and/or pre-embryo between the chambers.

2. The system according to claim 1, wherein the means for obtaining a mammalian oocyte is a system with a needle communicating under airtight conditions with a means for transferring from needle to said apparatus, such means for transferring comprises syringe and tube.

3. The system according to claim 1, wherein the means for obtaining a mammalian spermatozoa is a system in which the oxygen tension can be controlled.

4. The system according to claim 1, wherein the atmosphere within the chambers is kept aseptic.

5. The system according to claim 1, wherein the temperature of each chamber can be regulated independently.

6. The system according to claim 1, wherein the oxygen tension of each chamber is regulated independently by adding oxygen, nitrogen, carbon dioxide, helium or another inert gas, or a mixture of two or more of these gasses simultaneously

with removing gas from the chambers, in the way that the pressure of the air is in accordance with the atmosphere.

7. The system according to claim 6, wherein the pressure of the gasses inside the chambers is slightly higher than the pressure of the atmosphere surrounding the main chamber.

8. The system according to claim 1 to 7, wherein the humidity of each chamber can be controlled and regulated to a level between 50 and 100%.

9. The system according to claim 1, wherein said entrance port and said exit port is combined to a single opening means, such as a door.

10. The system according to claim 1, wherein said entrance port and said exit port is combined in a means for transporting cell culturing means and equipment to and from the outer chamber.

11. The system according to claim 10, wherein said combination of said entrance port and said exit port is an air lock.

12. The system according to claim 11, wherein said entrance port constitute an inner door of said air lock and said exit port constitute an outer door of said air lock.

13. The system according to claim 12, wherein said air lock comprises walls between said inner door and said outer door constituting a small air-tight chamber.

14. The system according to claim 13, wherein said inner door and said outer door only can be opened one at a time in the way that only one door can be open at a time, and the opening of one door can only set going when the other door is totally shut.

15. The system according to claim 14, wherein the atmosphere of said air lock can be controlled and adjusted including contents of oxygen, nitrogen, carbon dioxide, helium or another inert gas, temperature and humidity.

16. The system according to claim 15, wherein said inner door of said air lock only can open when the conditions including temperature, humidity and contents of oxygen is equal to the conditions inside the chamber which the air lock is positioned inside.

17. The system according to claim 1, wherein a microscope can be placed and used when handling the oocytes, spermatozoa and embryos.

18. The system according to claim 1 to 17, wherein a working area is obtained within said main chamber, said working area comprises a place for culturing means containing the cultured cell structures, where the cultured cell structures is observed in the microscope, and said working area comprises room for handling means.

19. The system according to claim 1 to 17, wherein a micro-insemination apparatus is placed within the main chamber

20. The system according to claim 1 to 19, wherein the main chamber comprises opening means permitting entrance to human to handle the cell culture or the equipment inside the chambers.

21. The system according to claim 20, wherein to the opening means is attached gloves. These gloves are mounted in the way that human hands can fit into the gloves and handling the cell culture or the equipment inside the chambers.

22. The system according to claim 20, wherein to the opening means is attached sticks, bars or instruments manipulated by fibre optics, by which the cell culture or the equipment can be handled.

23. The system according to claim 1, wherein the main chamber has at least one small part of its surface replaced with a membrane, said membrane is sterile and has a structure through which a needle can be stuck through, when the needle is removed said membrane fills up the area where the needle was stuck through, and no gasses or particles can diffuse through the membrane either when a

needle is stuck through the membrane or no needle is stuck through the membrane.

24. The system according to claim 1 to 23, wherein the at least two separate chambers are arranged as a main chamber and one or more smaller air-tight residence chambers.

25. The system according to claim 24, wherein said smaller residence chambers are located inside the main chamber or are attached to the main chamber.

26. The system according to claim 25, wherein, said residence chambers are air-tight and can be controlled independent of each other and independent of the main chamber according to temperature, humidity, and contents of oxygen, nitrogen and carbon dioxide.

27. The system according to claim 26, wherein said residence chambers constitute boxes for culture containers containing cell cultures of oocyte, spermatozoa, embryo, and stem cells including stem cell lines.

28. The system according to claim 27, each box is adapted for receiving one culture container containing the cell cultures of oocyte, spermatozoa, embryo, and stem cells including stem cell lines.

29. The system according to claim 28, wherein the number of said boxes correspond to the number of development stages of the of oocyte, spermatozoa, embryo and stem cells including stem cell lines.

30. The system according to claim 29, wherein said development stages comprises at least Immature oocyt, Mature oocyt, Spermatozoa, Fertilised oocyt, 4 cell embryo, 8 cell embryo, Morula, Blastocyst and stem cells including stem cell lines.

31. The system according to claim 1 to 30, wherein the oxygen tension and pressure of each chamber or air-tight boxes can be regulated by a computer by retrieving an image of the embryo in said chamber or said air-tight boxes.

32. The system according to claim 24, wherein said air-tight boxes is portable.

33. The system according to claim 32, wherein said air-tight boxes when removed from the apparatus can be connected to means for controlling temperature, humidity, and contents of oxygen, nitrogen and carbon dioxide.

34. The system according to claim 33, wherein said means for controlling temperature, humidity, and contents of oxygen, nitrogen and carbon dioxide is portable.

35. The system according to claim 32 to 34, wherein the wall of said boxes contain a membrane.

36. The system according to claim 32 to 35, wherein the small boxes comprises fastening means for fastening one or more cell culture containers.

37. The system according to claim 36, wherein the wall of said cell culture containers contain a sterile membrane.

38. The system according to claim 32 to 37, wherein the small boxes can be transported for at least 6 days.

39. The system according to claim 1, wherein the size of the main chamber constitute a room between 1 cm and 2 m of each wall.

40. Use of the system according to claim 1 to 39 for culturing cell cultures.

41. Use of the system according to claim 1 to 39 for culturing gametes, embryoes, blastocysts, stem cells, stem cell lines.

42. A method for in vitro producing a mammalian pre-embryo comprising the following steps:

a1) providing a mammalian oocyte,

a2) providing a mammalian spermatozoa,

b) culturing the oocyte and the spermatozoa,

c) fertilizing the oocyte with the spermatozoa obtaining a fertilized oocyte, and

d) allowing cell-division of the fertilized oocyte obtaining a multicellular pre-embryo,

wherein at least one of the steps a1) or a2) is conducted at an oxygen tension below 15 %.

43. The method according to claim 42, wherein the mammalian oocyte and mammalian spermatozoa are gametes obtained from female and male, respectively, of a mammal, such as a mammal selected from the group consisting of cows, pigs, horses, goats, sheep, dogs, cats, rabbits, rats, mice, tigers, lions, pandas, gorilla, whales, and humans.

44. The method according to claim 1, wherein the mammalian oocyte and mammalian spermatozoa are gametes obtained from cows.

45. The method according to claim 1, wherein the mammalian oocyte and mammalian spermatozoa are gametes obtained from pigs.

46. The method according to claim 1, wherein the mammalian oocyte and mammalian spermatozoa are gametes obtained from horses.

47. The method according to claim 1, wherein the mammalian oocyte and mammalian spermatozoa are gametes obtained from humans.

48. The method according to any of the preceding claims 1 to 47 , wherein the oocyte is obtained from the ovarium.

49. The method according to claim 48, wherein the oocyte is obtained from a primary ovarian follicle, secondary ovarian follicle, preantral ovarian follicle, early antral follicles or antral follicles.

50. The method according to claim 48 to 49, wherein the oocyte is obtained from the ovarium by aspiration into a needle.

51. The method according to claim 48 to 50, wherein the oocyte is obtained from the ovarium by removing part of or the entire ovarian tissue containing primary-, secondary or antral follicles and obtaining the containing primary-, secondary or antral follicles from said ovarian tissue.

52. The method according to claim 51, wherein the removed part or entire ovarian tissue has been subjected to freezing.

53. The method according to claim 50, wherein said needle is part of a syringe and the oxygen tension within said needle and said syringe is lower than 15%.

54. The method according to any of the preceding claims 1 to 53, wherein the oocyte is obtained from a mammal subsequent to treatment of said mammal with hormones capable of maturing oocytes.

55. The method according to claim 54, wherein said hormones is follicle-stimulating hormone (FSH).

56. The method according to claim 54, wherein said hormones is luteinizing hormone (LH).

57. The method according to claim 1 to 56, wherein the oocyte has been subjected to cooling.

58. The method according to claim 1 to 56, wherein the oocyte has been subjected to freezing.

59. The method according to claim 1 to 47, wherein the spermatozoa are immature spermatozoa or mature spermatozoa comprising spermatides or spermatocytes.

60. The method according to claim 59, wherein the immature or mature spermatozoa is obtained from the male under conditions of an oxygen tension below 15%.

61. The method according to claim 59 to 60, wherein the spermatozoa is obtained from testicular tissue removed from the male.

62. The method according to claim 61, wherein the testicular tissue has been subjected to freezing.

63. The method according to claim 59 to 62, wherein the spermatozoa is obtained from semen or testicular tissue.

64. The method according to claim 59 to 63, wherein the spermatozoa has been subjected to cooling.

65. The method according to claim 59 to 63, wherein the spermatozoa has been subjected to freezing.

66. The method according to claim 1 to 58, wherein the oocyte is an immature oocyte.

67. The method according to claim 66, wherein said immature oocyte is obtained from a follicle.

68. The method according to claim 67, wherein said follicle is between 1 and 25 mm in diameter, such as between 2 and 18 mm, such as between 3 and 13 mm, such as between 5 and 12 mm, such as between 7 and 11 mm, such as between 8 and 10 mm.

69. The method according to claim 66, wherein the immature oocyte is a primary oocyt.

70. The method according to claim 66, wherein the immature gamete is in the prophase of the first meiotic division.

71. The method according to claim 66, wherein the immature gamete is in the dictyotene stage of the first meiotic division.

72. The method according to claim 66, wherein the immature gamete is in the late stage of the first meiotic metaphase.

73. The method according to claim 66 to 72, wherein culturing of the immature gamete up to metaphase II is associated with a synchronised cumulus-, cytoplasm-, and nuclear maturation.

74. The method according to any of claims 66 to 73, wherein culturing of the immature gamete up to metaphase II is completed within a period of 20 to 30 hours.

75. The method according to any of claims 66 to 74, wherein step b) includes a step for culturing said immature oocyte under conditions allowing maturation of the oocyte.

76. The method according to claim 75, wherein the conditions include an oxygen tension below 15 %.

77. The method according to any of the preceding claims 1 to 76, wherein the oxygen tension is below 13 %, such as below 11 %, such as below 10 %, such as below 9 %, such as below 8 %, such as below 7 %, such as below 6 %, such as below 5 %, such as below 4 %, such as below 3 %, such as below 2 %, such as below 1 %.

78. The method according to claim 1, wherein step b) comprises co-culturing the oocyte and the spermatozoa.

79. The method according to claim 78, wherein the oocyte and the spermatozoa is co-cultured for at least 1 minute, such as at least 2 minutes, such as at least 5 minutes, such as at least 10 minutes, such as at least 30 minutes, such as at

least 1 hour, such as at least 2 hours, such as at least 3 hours, such as at least 4 hours, such as at least 5 hours, such as at least 10 hours, such as at least 15 hours, such as at least 20 hours, such as at least 25 hours, such as at least 30 hours, such as at least 35 hours, such as at least 40 hours, such as at least 45 hours, such as at least 50 hours.

80. The method according to claim 78 to 79, wherein the oocyte and the spermatozoa is co-cultured with feeder-cells.

81. The method according to claim 78 to 80, wherein the co-culturing of oocyte and spermatozoa results in fertilization of oocyte by spermatozoa.

82. The method according to claim 1 to 77, wherein the oocyte is fertilised with the spermatozoa by Intracytoplasmic sperm injection (ICSI).

83. The method according to any of the preceding claims 1 to 82, wherein the fertilised oocyte is cultured to an embryo stage ready for transfer to the female uterus.

84. The method according to claim 83, wherein the stage for transfer of the embryo is obtained by least ½ day culture following fertilisation of the oocyte, such as at least 1 day, for example at least 2 days, such as at least 3 days, for example at least 4 days, such as at least 5 days, such as at least 6 days, for example at least 7 days, such as at least 8 days, for example at least 9 days.

85. The method according to claim 83 to 84, wherein the embryo stage is the two-cell stage.

86. The method according to claim 83 to 84, wherein the embryo stage is the four-cell stage.

87. The method according to claim 83 to 84, wherein the embryo stage is the six-cell stage.

88. The method according to claim 83 to 84, wherein the embryo stage is the eight-cell stage.

89. The method according to claim 83 to 84, wherein the embryo stage is the morula stage.

90. The method according to claim 83 to 84, wherein the embryo stage is the blastocyst stage.

91. The method according to claim 83 to 84, wherein the embryo stage is the blastocyst stage, where zona pellucida is disappeared.

92. The method according to claim 85 to 91, wherein the zona pellucida of said embryo is opened to help the embryo hatch before implantation into the uterus.

93. The method according to claim 92, wherein the zona pellucida is opened by assisted hatching using either laser, mechanical force or acid tyrode.

94. The method according to claim 83 to 93, wherein fragments of cell debris are removed from said embryo.

95. The method according to any of the preceding claims 1 to 94, wherein at least a part of step a) and at least one of the other steps are conducted at an oxygen tension below 15 %.

96. The method according to any of the preceding claims 1 to 95, wherein at least 3 of the steps a), b), c) and d) are conducted at an oxygen tension below 15 %.

97. The method according to any of the preceding claims 1 to 96, wherein all of the steps a), b), c) and d) are conducted at an oxygen tension below 15 %.

98. The method according to any of the preceding claims 1 to 97, wherein the oxygen tension of step d) is higher as compared to the oxygen tension of any of the other steps b) and c).

99. The method of claim 66 to 76, wherein the conditions allowing maturation of the oocyte includes a rise in the oxygen tension followed by lowering the oxygen tension.

100. The method of claim 99, wherein the rise of the oxygen tension is at least 1 unit, such as at least 2 units, for example at least 3 units, such as at least 4 units, for example at least 5 units, such as at least 6 units, for example at least 7 units, such as at least 8 units, for example at least 9 units, such as at least 10 units.

101. The method according to any of the preceding claims 1 to 100, wherein the oxygen tension is at least 0,5 % and maximum 21 %.

102. The method of claim 99 to 100, wherein the rise of the oxygen tension is maintained for at least 5 minutes, such as at least 10 minutes, for example at least 20 minutes, such as at least 30 minutes, such as at least 45 minutes, for example at least 1 hour, such as at least 1½ hour, for example at least 2 hours, such as at least 2½ hours, for example at least 3 hours, such as at least 4 hours, for example at least 5 hours, such as at least 6 hours, for example at least 7 hours, such as at least 8 hours, for example at least 9 hours, such as at least 10 hours.

103. The method according to any of the preceding claims, 1 to 102 wherein the oxygen tension is regulated in accordance to the phase and the condition of the oocyte or the embryo.

104. The method according to any of the preceding claims 1 to 103, wherein the culturing conditions of oocyte, spermatozoa and embryo comprises a temperature of 30-45 °C, such as 32-42 °C, such as 34-40 °C, such as 36-38 °C, such as 36,5-37,5 °C, such as about 37 °C.

105. The method according to any of the preceding claims 1 to 104, wherein the culturing conditions of oocyte, spermatozoa and embryo comprises a temperature of about 37 °C.

106. The method according to any of the preceding claims 1 to 105, wherein the oxygen tension of the culture is regulated by adding oxygen, nitrogen, carbon dioxide, helium or another inert gas or a mixture of two or more of these gasses to the environment of the in vitro culture.

107. The method according to any of the preceding claims 1 to 107, wherein all of the steps a), b), c) and d) and the transfer of the embryo to the uterus are conducted at an oxygen tension below 20 %, such as below 15%, such as below 13 %, such as below 11 %, such as below 10 %, such as below 9 %, such as below 8 %, such as below 7 %, such as below 6 %, such as below 5 %, such as below 4 %, such as below 3 %, such as below 2 %, such as below 1 %.

108. The method according to any of the preceding claims 1 to 108, wherein all of the steps a), b), c) and d) and the transfer of the embryo to the uterus are conducted under aseptic conditions.

109. The method according to any of the preceding claims 1 to 108, wherein the medium contains lipid or lipid precursor, such as sterol or functionally equivalents derivatives thereof.

110. The method according to any of the preceding claims 1 to 109, wherein the medium among other factors contains ATA (Aurin Tricarboxylic Acid).

111. The method according to any of the preceding claims 1 to 110, wherein the additives are Medi-Cult SSR 4x, Medi-Cult SSR 4xa, Medi-Cult SSR 4xb, Medi-Cult SSR2, or Medi-Cult SSR3.

112. The method according to any of the preceding claims 1 to 111, wherein the culturing media for each stage of development of oocyte or spermatozoa comprises a medium known to persons skilled in the art.

113. The method according to claim 112, wherein the medium is modified to the conditions of lowered oxygen tension. The modification may be physical of

chemical, in the latter case e.g. by utilising an oxygen molecule carrier or a catalase.

114. The method according to any of claim 1 to 113, wherein the culture conditions provide formation of an increased number of inner cells of the embryo.

115. The method according to any of claim 1 to 114, wherein the culturing of the embryo result in an embryo with 3-5 blastomers and the score selected among 1.0; 2.0; 2.1 and 2.2

116. The method according to any of claim 1 to 114, wherein the culturing of the embryo result in an embryo with 4 blastomers and the score selected among 1.0; 2.0 and 2.1.

117. The method according to any of claim 1 to 114, wherein the culturing of the embryo result in an embryo at the 3-5 cell stage with 10- 50% fragmentation, thus the embryo is awarded 6-7 points in a CES scoring system.

118. The method according to any of claim 1 to 114, wherein the culturing of the embryo result in an embryo at the 3-5 cell stage with less than 10% fragmentation, thus the embryo is awarded 7-8 points in a CES scoring system.

119. The method according to any of claim 1 to 114, wherein the culturing of the embryo result in an embryo at the 4 cell stage with less than 10% fragmentation, thus the embryo is awarded 8 points in a CES scoring system.

120. The method according to any of claim 1 to 114, wherein the culturing of the embryo result in an embryo of 7-9 cells 64-67 hours after fertilisation and obtain the score of 60-100 according to the GES scoring system.

121. The method according to any of claim 1 to 114, wherein the culturing of the embryo result in an embryo of 7 cells, grade I or 8 cells, grade I or 8 cells, grade II or 9 cells, grade I 64-67 hours after fertilisation and obtain the score of 70-100 according to the GES scoring system.

122. The method according to any of claim 1 to 114, wherein the culturing of the embryo result in an embryo of 7 cells, grade I or 8 cells, grade I or 8 cells, grade II or 9 cells, grade I 64-67 hours after fertilisation and obtain the score of 80-100 according to the GES scoring system.

123. A method for implanting a pre-embryo, comprising culturing oocyte and spermatozoa as defined in any of claims 1 to 122, and transferring the resulting pre-embryo to the uterus of a mammalian female.

124. The method according to claim 123, wherein said embryo is transferred to the uterine tube of the female uterus.

125. The method according to claim 123 to 124 wherein said embryo is transferred to the uterus of a female following a hormone treatment of the female.

126. A method for in vitro producing a mammalian pre-embryo comprising the following steps:

a) providing gametes selected from a mammalian oocyte and a mammalian spermatozoa,

b) culturing the oocyte and the spermatozoa,

c) fertilizing the oocyte with the spermatozoa obtaining a fertilized oocyte, and

e) allowing cell-division of the fertilized oocyte obtaining a multicellular pre-embryo,

wherein the culture is performed at an oxygen tension allowing cultivation of the cells and wherein at least one of the steps comprises a change in the oxygen tension.

127. The method according to claim 126, wherein the mammalian oocyte and mammalian spermatozoa are gametes obtained from female and male, respectively, of a mammal, such as a mammal selected from the group consisting of cows, pigs, horses, goats, sheep, dogs, cats, rabbits, rats, mice, tigers, lions, pandas, gorilla, whales, and humans.

128. The method according to claim 126, wherein the mammalian oocyte and mammalian spermatozoa are gametes obtained from cows.

129. The method according to claim 126, wherein the mammalian oocyte and mammalian spermatozoa are gametes obtained from pigs.

130. The method according to claim 126, wherein the mammalian oocyte and mammalian spermatozoa are gametes obtained from horses.

131. The method according to claim 126, wherein the mammalian oocyte and mammalian spermatozoa are gametes obtained from humans.

132. The method according to any of the preceding claims 126 to 131, wherein the oocyte is obtained from the ovarium.

133. The method according to claim 132, wherein the oocyte is obtained from a primary ovarian follicle, secondary ovarian follicle, preantral ovarian follicle, early antral follicles or antral follicles.

134. The method according to claim 132 to 133, wherein the oocyte is obtained from the ovarium by aspiration into a needle.

135. The method according to claim 132 to 134, wherein the oocyte is obtained from the ovarium by removing part of or the entire ovarian tissue containing primary-, secondary or antral follicles and obtaining the containing primary-, secondary or antral follicles from said ovarian tissue.

136. The method according to claim 135, wherein the removed part or entire ovarian tissue has been subjected to freezing.

137. The method according to claim 134, wherein said needle is part of a syringe and the oxygen tension within said needle and said syringe is lower than 15%.

138. The method according to any of the preceding claims 126 to 137, wherein the oocyte is obtained from a mammal subsequent to treatment of said mammal with hormones capable of maturing oocytes.

139. The method according to claim 138, wherein said hormones is follicle-stimulating hormone (FSH).

140. The method according to claim 138, wherein said hormones is luteinizing hormone (LH).

141. The method according to claim 126 to 140, wherein the oocyte has been subjected to cooling.

142. The method according to claim 126 to 140, wherein the oocyte has been subjected to freezing.

143. The method according to claim 126 to 131, wherein the spermatozoa are immature spermatozoa or mature spermatozoa comprising spermatides or spermatocytes.

144. The method according to claim 143, wherein the immature or mature spermatozoa is obtained from the male under conditions of an oxygen tension below 15%.

145. The method according to claim 143 to 144, wherein the spermatozoa are obtained from testicular tissue removed from the male.

146. The method according to claim 145, wherein the testicular tissue has been subjected to freezing.

147. The method according to claim 143 to 146, wherein the spermatozoa is obtained from semen or testicular tissue.

148. The method according to claim 143 to 147, wherein the spermatozoa has been subjected to cooling.

149. The method according to claim 143 to 147, wherein the spermatozoa has been subjected to freezing.

150. The method according to claim 126 to 142, wherein the oocyte is an immature oocyte.

151. The method according to claim 150, wherein said immature oocyte is obtained from a follicle.

152. The method according to claim 151, wherein said follicle is between 1 and 25 mm in diameter, such as between 2 and 18 mm, such as between 3 and 13 mm, such as between 5 and 12 mm, such as between 7 and 11 mm, such as between 8 and 10 mm.

153. The method according to claim 150, wherein the immature oocyte is a primary oocyt.

154. The method according to claim 150, wherein the immature gamete is in the prophase of the first meiotic division.

155. The method according to claim 150, wherein the immature gamete is in the dictyotene stage of the first meiotic division.

156. The method according to claim 150, wherein the immature gamete is in the late stage of the first meiotic metaphase.

157. The method according to claim 150 to 156, wherein culturing of the immature gamete up to metaphase II is associated with a synchronised cumulus-, cytoplasm-, and nuclear maturation.

158. The method according to any of claims 150 to 157, wherein culturing of the immature gamete up to metaphase II is completed within a period of 20 to 30 hours.

159. The method according to claim 126 to 158, wherein a drop in the oxygen tension is performed in one of the steps a), b), c) or e) under conditions allowing maturation of the oocyte or spermatozoa.

160. The method according to claim 126 to 158, wherein a drop in the oxygen tension is performed in two of the steps a), b), c) or e) under conditions allowing maturation of the oocyte or spermatozoa.

161. The method according to claim 126 to 158, wherein a drop in the oxygen tension is performed in three of the steps a), b), c) or e) under conditions allowing maturation of the oocyte or spermatozoa.

162. The method according to claim 126 to 158, wherein a drop in the oxygen tension is performed in all of the steps a), b), c) or e) under conditions allowing maturation of the oocyte or spermatozoa.

163. The method according to claim 126 to 158, wherein a rise in the oxygen tension is performed in one of the steps a), b), c) or e) under conditions allowing maturation of the oocyte or spermatozoa.

164. The method according to claim 126 to 158, wherein a rise in the oxygen tension is performed in two of the steps a), b), c) or e) under conditions allowing maturation of the oocyte or spermatozoa.

165. The method according to claim 126 to 158, wherein a rise in the oxygen tension is performed in three of the steps a), b), c) or e) under conditions allowing maturation of the oocyte or spermatozoa.

166. The method according to claim 126 to 158, wherein a rise in the oxygen tension is performed in all of the steps a), b), c) or e) under conditions allowing maturation of the oocyte or spermatozoa.

167. The method according to any of the preceding claims 126 to 166, wherein the initially oxygen tension of the culture is selected to a level between 1% and 21%.

168. The method according to any of the preceding claims 126 to 167, wherein the change in the oxygen tension is a change to an oxygen tension below 20 %, for example below 19%, such as below 18%, for example below 17%, such as below 16 %, for example below 15%, such as below 14%, for example below 13%, such as below 12 %, for example below 11 %, such as below 10 %, for example below 9 %, such as below 8 %, for example below 7 %, such as below 6 %, for example below 5 %, such as below 4 %, for example below 3 %, such as below 2 %, for example below 1 %.

169. The method according to any of the preceding claims 126 to 168, wherein the change in the oxygen tension is a change in the oxygen tension where it is lowered by at least 1 unit, such as at least 2 units, for example at least 3 units, such as at least 4 units, for example at least 5 units, such as at least 6 units, for example at least 7 units, such as at least 8 units, for example at least 9 units, such as at least 10 units, for example at least 11 units, such as at least 12 units, for example at least 13 units, such as at least 14 units, for example at least 15 units, such as at least 16 units, for example at least 17 units, such as at least 18 units, for example at least 19 units.

170. The method according to any of the preceding claims 126 to 169, wherein the change in the oxygen tension is a change in the oxygen tension where it is lowered by 1-19 units, such as 1-18 units, for example 1-17 units, such as 1-16 units, for example 1-15 units, such as 1-14 units, for example 1-13 units, such as 1-12 units, for example 1-11 units, such as 1-10 units, for example 1-9 units, such as 1-8 units, for example 1-7 units, such as 1-6 units, for example 1-5 units, such as 1-4 units, for example 1-3 units, such as 1-2.

171. The method according to any of the preceding claims 126 to 170, wherein the change in the oxygen tension is a change to an oxygen tension above 2%, for example above 3%, such as above 4%, for example above 5%, such as above 6%, for example above 7%, such as above 8%, for above 9%, such as above 10%, for example above 11%, such as above 12%, for example above 13%, such as above 14%, for example above 15%, such as above 16%, for example above 17%, %, such as above 18%, for example above 19%, such as above 20%, for example above 21%.

172. The method according to any of the preceding claims 126 to 171, wherein the change in the oxygen tension is a change in the oxygen tension where it is increased by at least 1 unit, such as at least 2 units, for example at least 3 units, such as at least 4 units, for example at least 5 units, such as at least 6 units, for example at least 7 units, such as at least 8 units, for example at least 9 units, such as at least 10 units, for example at least 11 units, such as at least 12 units, for example at least 13 units, such as at least 14 units, for example at least 15 units, such as at least 16 units, for example at least 17 units, such as at least 18 units, for example at least 19 units.

173. The method according to any of the preceding claims 126 to 172, wherein the change in the oxygen tension constitute an increasement by 1-3 units, such as 2-5 units, for example 3-7 units, such as 4-9 units, for example 5-11 units, such as 6-13 units, for example 8-15 units, such as 10-18 units, for example 12-20 units.

174. The method according to any of the preceding claims 126 to 173, wherein the oxygen tension followed a change is regulated to a higher or lower level compared to the initially oxygen tension.

175. The method according to claim 174, where the oxygen tension followed the change can be regulated to a level between 1% and 21%.

176. The method according to any of the preceding claims 126 to 175, wherein the change in the oxygen tension is conducted for at least 1 minute, for example at least 2 minutes, such as at least 3 minutes, for example at least 4

minutes, such as at least 5 minutes, for example at least 6 minutes, such as at least 7 minutes, for example at least 8 minutes, such as at least 9 minutes, such as at least 10 minutes, for example at least 11 minutes, such as at least 12 minutes, for example at least 13 minutes, such as at least 14 minutes, for example at least 15 minutes, such as at least 16 minutes, for example at least 20 minutes, such as at least 30 minutes, such as at least 45 minutes, for example at least 1 hour, such as at least 1½ hour, for example at least 2 hours, such as at least 2½ hours, for example at least 3 hours, such as at least 4 hours, for example at least 5 hours, such as at least 6 hours, for example at least 7 hours, such as at least 8 hours, for example at least 9 hours, such as at least 10 hours.

177. The method according to any of the preceding claims 126 to 176, wherein step b) comprises co-culturing the oocyte and the spermatozoa.

178. The method according to claim 177, wherein the oocyte and the spermatozoa is co-cultured for at least 1 minute, such as at least 2 minutes, such as at least 5 minutes, such as at least 10 minutes, such as at least 30 minutes, such as at least 1 hour, such as at least 2 hours, such as at least 3 hours, such as at least 4 hours, such as at least 5 hours, such as at least 10 hours, such as at least 15 hours, such as at least 20 hours, such as at least 25 hours, such as at least 30 hours, such as at least 35 hours, such as at least 40 hours, such as at least 45 hours, such as at least 50 hours.

179. The method according to claim 177 to 178, wherein the oocyte and the spermatozoa is co-cultured with feeder-cells.

180. The method according to claim 177 to 179, wherein the co-culturing of oocyte and spermatozoa results in fertilization of oocyte by spermatozoa.

181. The method according to claim 126 to 176, wherein the oocyte is fertilised with the spermatozoa by Intracytoplasmic sperm injection (ICSI).

182. The method according to any of the preceding claims 126 to 181, wherein the fertilised oocyte is cultured to an embryo stage ready for transfer to the female uterus.

183. The method according to claim 182, wherein the stage for transfer of the embryo is obtained at least ½ day culture following fertilisation of the oocyte, such as at least 1 day, for example at least 2 days, such as at least 3 days, for example at least 4 days, such as at least 5 days, such as at least 6 days, for example at least 7 days, such as at least 8 days, for example at least 9 days.

184. The method according to claim 182 to 183, wherein the embryo stage is the two-cell stage.

185. The method according to claim 182 to 183, wherein the embryo stage is the four-cell stage.

186. The method according to claim 182 to 183, wherein the embryo stage is the six-cell stage.

187. The method according to claim 182 to 183, wherein the embryo stage is the eight-cell stage.

188. The method according to claim 182 to 183, wherein the embryo stage is the morula stage.

189. The method according to claim 182 to 183, wherein the embryo stage is the blastocyst stage.

190. The method according to claim 182 to 183, wherein the embryo stage is the blastocyst stage, where zona pellucida is disappeared.

191. The method according to claim 184 to 190, wherein the zona pellucida of said embryo is opened to help the embryo hatch before implantation into the uterus.

192. The method according to claim 191, wherein the zona pellucida is opened by assisted hatching using either laser, mechanical force or acid tyrode.

193. The method according to claim 182 to 192, wherein fragments of cell debris are removed from said embryo.

194. The method according to any of the preceding claims 126 to 193, wherein the oxygen tension is at least 0,5 % and maximum 21%.

195. The method according to any of the preceding claims 126 to 194, wherein the oxygen tension is regulated in accordance to the phase and the condition of the oocyte or the embryo.

196. The method according to any of the preceding claims 126 to 195, wherein the culturing conditions of oocyte, spermatozoa and embryo comprises a temperature of 30-45 degree C, such as 32-42 degree C, such as 34-40 degree C, such as 36-38 degree C, such as 36,5-37,5 degree C, such as about 37 degree C.

197. The method according to any of the preceding claims 126 to 196, wherein the culturing conditions of oocyte, spermatozoa and embryo comprises a temperature of about 37 degree C.

198. The method according to any of the preceding claims 126 to 197, wherein the oxygen tension of the culture is regulated by adding oxygen, nitrogen, carbon dioxide, helium or another inert gas or a mixture of two or more of these gasses to the environment of the in vitro culture.

199. The method according to any of the preceding claims 126 to 198, wherein all of the steps a), b), c) and e) and the transfer of the embryo to the uterus are conducted under aseptic conditions.

200. The method according to any of the preceding claims 126 to 199, wherein the medium contains lipid or lipid precursor, such as sterol or functionally equivalents derivatives thereof.

201. The method according to any of the preceding claims 126 to 200, wherein the medium among other factors contains ATA (Aurin Tricarboxylic Acid).

202. The method according to any of the preceding claims 126 to 201, wherein the additives are Medi-Cult SSR 4x, Medi-Cult SSR 4xa, Medi-Cult SSR 4xb, Medi-Cult SSR2, or Medi-Cult SSR3.

203. The method according to any of the preceding claims 126 to 202, wherein the culturing media for each stage of development of oocyte or spermatozoa comprises a medium known to persons skilled in the art.

204. The method according to claim 203, wherein the medium is modified to the conditions of lowered oxygen tension. The modification may be physical of chemical, in the latter case e.g. by utilising an oxygen molecule carrier or a catalase.

205. The method according to any of claim 126 to 204, wherein the culture conditions provide formation of an increased number of inner cells of the embryo.

206. The method according to any of claim 126 to 205, wherein the culturing of the embryo result in an embryo with 3-5 blastomers and the score selected among 1.0; 2.0; 2.1 and 2.2

207. The method according to any of claim 126 to 205, wherein the culturing of the embryo result in an embryo with 4 blastomers and the score selected among 1.0; 2.0 and 2.1.

208. The method according to any of claim 126 to 205, wherein the culturing of the embryo result in an embryo at the 3-5 cell stage with 10- 50% fragmentation, thus the embryo is awarded 6-7 points in a CES scoring system.

209. The method according to any of claim 126 to 205, wherein the culturing of the embryo result in an embryo at the 3-5 cell stage with less than 10% fragmentation, thus the embryo is awarded 7-8 points in a CES scoring system.

210. The method according to any of claim 126 to 205, wherein the culturing of the embryo result in an embryo at the 4 cell stage with less than 10% fragmentation, thus the embryo is awarded 8 points in a CES scoring system.

211. The method according to any of claim 126 to 205, wherein the culturing of the embryo result in an embryo of 7-9 cells 64-67 hours after fertilisation and obtain the score of 60-100 according to the GES scoring system.

212. The method according to any of claim 126 to 205, wherein the culturing of the embryo result in an embryo of 7 cells, grade I or 8 cells, grade I or 8 cells, grade II or 9 cells, grade I 64-67 hours after fertilisation and obtain the score of 70-100 according to the GES scoring system.

213. The method according to any of claim 126 to 205, wherein the culturing of the embryo result in an embryo of 7 cells, grade I or 8 cells, grade I or 8 cells, grade II or 9 cells, grade I 64-67 hours after fertilisation and obtain the score of 80-100 according to the GES scoring system.

214. A method for implanting a pre-embryo, comprising culturing oocyte and spermatozoa as defined in any of claims 126 to 213, and transferring the resulting pre-embryo to the uterus of a mammalian female.

215. The method according to claim 215, wherein said embryo is transferred to the uterine tube of the female uterus.

216. The method according to claim 215 to 216 wherein said embryo is transferred to the uterus of a female following a hormone treatment of the female.

217. A method of producing a stem cell, said method comprises:

a) Providing a multicellular pre-embryo as defined according to any of claim 42 to 213,
b) isolating a multicellular pre-embryo of a),
c) isolating cells from the inner cell mass of the pre-embryo of b),
d) culturing said isolated cells from the inner cell mass in a matrix gel,
e) obtaining stem cells.

218. The method according to claim 217, wherein at least one of the steps b), c), d), and e) is conducted at an oxygen tension below 15%.

219. The method according to claim 217, wherein at least two of the steps b), c), d), and e) are conducted at an oxygen tension below 15%.

220. The method according to claim 217, wherein at least three of the steps b), c), d), and e) are conducted at an oxygen tension below 15%.

221. The method according to claim 217, wherein all of the steps b), c), d), and e) are conducted at an oxygen tension below 15%.

222. A stem cell obtained from any of the methods of claim 217 to 221.

223. A stem cell according to claim 222, wherein said stem cell is stabile in the sense no mutations or other genetic changes occur within the chromosomes or antigenesity on the surfaces of the cells.

224. A stem cell line obtained from the stem cells of claim 217 to 221.

225. Use of a stem cell obtained from the method of claim 217 to 221 to produce a stem cell line.

226. A stem cell package comprising:

- Stem cells as defined in any of claims 217 to 221,
- Certificate describing the culture conditions for the stem cells and the cell cultures from which said stem cells are obtained.